THE INDUCTION OF SUPPRESSION TO NUCLEOSIDE ANTIGENS

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DEDICATED TO

MY LOVING

MOTHER AND FATHER

AND

KAREN

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INTRODUCTION

Tolerance is a term that has been operationally defined as immunological unresponsiveness to an antigen against which an immune response would normally be mounted, and used in this manner encompasses a variety of possible mechanisms. One such mechanism is carrier-induced tolerance, which in the following discussion specifically refers to tolerance induced by hapten coupled to isologous serum proteins and is contrasted with suppression, which induces tolerance through the mechanism of suppressor T cells generated by presenting hapten on cell surfaces.

In this thesis, a new model of suppression is described, in which the concept of cell-bound hapten has been extended to autoantigens. Nucleosides covalently coupled to spleen cells were used to generate suppression of anti-nucleoside antibody production. The data to be presented suggest that the mechanism involves the induction of T lymphocyte mediated suppression. This model is significant in being the first to utilize a naturally occurring hapten and is of direct relevance to the study of autoimmune disease, specifically systemic lupus erythematosus (SLE) a disease whose pathology is caused by the production of autoantibody to nucleic acids and resultant tissue damage from immune complex deposition (88). One of the possibilities proposed for the pathogenesis of SLE is the loss of suppressor cells against autoantigen resulting in autoantigen production. A model in which suppressor cells can be raised against the immune response to the autoantigen, specifically

nucleoside, will enable this hypothesis to be tested. Most importantly, the generation of suppression of the antibody response to nucleic acids might be of clinical significance in the management and treatment of SLE.

The next section consists of a broad survey of suppressor T cells in general followed by a more specific discussion of the major hapten-specific suppressor systems in the literature. The final section of this introduction is a brief description of the rationale and objectives of this thesis project.

BACKGROUND OF SUPPRESSOR T CELLS

Since the concept of suppressor lymphocyte activity was first introduced by Gershon in 1970 (1), the suppressor T cell (T_s) has been demonstrated in the regulation of nearly every aspect of the immune response, and has been implicated in a wide range of clinical disorders. It is now well documented that the suppressor T cell is a distinct subset of T lymphocytes characterized by unique, genetically determined cell surface antigens (thy 1^+ ly $2,3^+$ I-J^+), distinguishing it from the helper T cell (thy 1^+ ly 1^+ I-J^-) and the cytotoxic T cell (thy 1^+ ly $2,3^+$ I-J^-) (2-4). Another cell surface marker on the T_s membrane appears to be a histamine receptor (9,10). Suppressor T cells are relatively more immature, functionally short-lived and spleen seeking, whereas helper T cells are more mature, long-lived and migrate preferentially to the lymph nodes (5,6).

The mechanism by which the T_s mediates suppression is still controversial although it appears that both antigen-specific and non-specific soluble "suppressor factors" are elaborated in most suppressor systems studied. The target cell of suppressor factor is also controversial with data supporting B cells (7), T cells (11) and macrophages (72). The best characterization of suppressor factor is by Taussig and Holliman who recently produced a suppressor factor specific for the antibody response to sheep erythrocytes utilizing the technique of a hybrid T-cell line (114). This factor is specific for SRBC but can also suppress the response to a hapten

coupled to the SRBC. The molecule is non-immunoglobulin, composed of a large chain and a small chain in non-covalent association with a native molecular weight of 200,000 (115). The large chain binds SRBC and the small chain binds H-2 determinants (115).

T cell mediated suppression may be classified into two general categories: suppression induced by extrinsic antigen, and suppression induced by autologous antigen anti-immunoglobulin with specificity for host allotypic and idiotypic determinants (7,8). Extrinsically induced suppression may be either antigen specific, affecting only the immune responses directed toward that antigen, or non-specific, suppressing the immune responses to multiple unrelated antigens (8).

Antigen-specific T cell suppression has been demonstrated in a large number of immunologic phenomena: the regulation of antibody production e.g. IgG (11), IgE (12-14), IgM (15), and antibody responses under Ir gene control e.g. nonresponders to the terpolymer antigen L-glutamic acid-L-alanine-L-tyrosine (GAT) (16, 17, 18). Specific suppression has been demonstrated in low zone tolerance (19) and high zone tolerance (20). Antigen specificity has also been found in the suppression of cell-mediated immunity. The role of T_s in blocking the afferent and/or efferent limb of contact sensitivity has been extensively studied in the picryl chloride (TNCB) system (21,22,23) and the dinitrofluorobenzene (DNFB) system (24,25,26). These experimental models will be discussed in detail below. Delayed

type hypersensitivity induced by sheep erythrocytes (27), murine lymphocytic choriomeningitis virus (28), fowl gammaglobulin (29) and the hapten azobenzene arsonate (ABA) (30) have all been shown to be subject to T_s suppression. The graft versus host reaction has been suppressed by T_s in several systems when the introduction of immunocompetent cells into histoincompatible and immunodeficient hosts is accompanied by an injection of spleen cells or thymus cells (31,32). Host responsiveness to various murine tumors: methylcholanthrene-induced fibrosarcoma (33), Walker lung carcinoma (34,35), mastocytoma (36), ultraviolet-induced fibrosarcoma (37) and others (38) are suppressed by tumor induced suppressor T cells.

T cell mediated suppression has been demonstrated to be non-antigen specific in a variety of instances. The phenomenon of antigenic competition, in which the induction of an immune response to one antigen non-specifically interferes with a subsequent immune response to second, unrelated antigen (39), has been shown to be thymus dependent (40). Antigenic competition, mediated by T_s, can suppress antibody responses (6), prevent the development of systemic graft versus host reactions (5), suppress allograft and tumor rejection (8), and mixed lymphocyte reactions (41), and can also cause acute anergy in delayed hypersensitivity (6). Suppressor T cells activated by mitogens such as concanavalin A (Con A) are able to suppress both cell mediated and humoral responses non-specifically (42-44). Once activated, the radiosensensitive precursors of T_s become

radioresistant (45). Activation of T_s requires protein synthesis, oxidative phosphorylation and an intact microtubule system (46). Non-specific T_s have also been demonstrated in spleen cells of neonatal (approximately 14 days) and young mice, and unprimed thymus cells (5,6). It has become evident that the development and expression of autoimmunity is under the regulation of non-antigen specific T cell suppression (6,47,48).

A number of autoimmune disorders have been studied, both clinically and in animal and experimental models. The classic animal model of autoimmune disease is the New Zealand black (NZB) mouse which develops a syndrome of autoimmune hemolytic anemia, and glomerulonephritis closely resembling human systemic lupus erythematosus (SLE) (49). The immune system of the young NZB mice is mature compared to other strains and is characterized by excessive antibody production to some antigens and early loss of tolerance to foreign and self antigens (6). Adult NZB mice have high titers of thymocytotoxic antibody (6) and a markedly reduced population of T lymphocytes resulting in unusual viral infections, malignancies, and decreased capacity for tumor and graft rejection as well as decreased GVH response. In effect, the NZB mouse is characterized by excessive B cell function and deficient T cell function. A large body of evidence suggests that the loss of suppressor T cell function leads to the onset of the autoimmune disease: many of the T cell defects are corrected by replenishing the adult NZB mice with T cells from young NZB mice (47). Neonatal thymectomy

accelerates the course of autoimmune disease (6). Infusion of thymosin into young mice delays the appearance of autoantibodies (50). Other autoimmune disorders have been studied with similar associations between the loss of T_s and the development of autoimmune disease: Hashimoto's thyroiditis (51,52), experimental autoimmune encephalomyelitis (53), myasthenia gravis (54), experimental autoimmune hemolytic anemia (55), human SLE (56,57) and juvenile rheumatoid arthritis (58).

Whereas autoimmune disorders may in part be due to a deficiency of T_s, there is now evidence that in contrast, immunodeficiency disorders may be related to an abnormally high number of T_s: common variable hypogammaglobulinemia (59), selective IgA deficiency (59), "infectious agammaglobulinemia" of birds (60).

The current interest in suppressor cells have resulted in many other reports of associations between clinical syndromes and diseases, and suppressor cells: aplastic anemia (61), fungal infections (62), post-traumatic immunosuppression (63), tropical splenomegaly syndrome (64), melanoma associated immunosuppression (65), inflammatory bowel disease (66), sarcoidosis (67), diabetes (68), and pregnancy (69).

Having discussed both specific and non-specific antigen dependent T cells mediated suppression, we now turn to T_s suppression of antibody directed against autologous antigen - anti-immunoglobulin. These T_s have specificity for determinants on immunoglobulin, both allotypic (70) and idiotypic (71), and

are generated by the exposure of the animals (neonates) to anti-immunoglobulin specific for the host allotype or idiotype. That T_s cell can suppress idiotype production is advantageous because it allows the regulation of immune reactivity to remain even after the antigen to which the idiotype was directed, has been catabolized and cleared. Idiotype specific T_s can be induced by residual antibody and the host will be able to exert negative control on antibody production without the continued presence of antigen (7).

HAPTEN SPECIFIC SUPPRESSOR T CELLS

Hapten-specific suppression has been investigated extensively in the picryl chloride (trinitrophenyl) system and the DNFB (dinitrophenyl) system mentioned above as contact sensitivity systems in which the mechanism of tolerance has been shown to involve T_s mediated suppression. These systems, as well as several others, will be presented in greater detail and compared with each other, with regard to the following criteria: the hapten, its characteristics and mode of presentation, dosage requirement, the kinetics of suppression, specificity, transfer suppression and genetic restrictions.

Hapten coupled cells were first used by Battisto and Bloom in 1966 to induce tolerance in guinea pigs to picryl chloride (trinitrochlorobenzene) (81). Intravenous injections were made of picryl chloride coupled to syngeneic spleen cells, heat-killed spleen cells, erythrocytes and erythrocyte membranes (81).

All forms of membrane-coupled hapten suppressed development of delayed hypersensitivity and passive cutaneous anaphylaxis antibody formation (81).

The picryl chloride system was investigated further by Asherson and Zembala. Their contact sensitivity system consists of sensitizing the skin of TNCB mice with picryl chloride and challenging 4-5 days later (22). Tolerance is produced by multiple i.v. injectionsof picryl sulphonic acid (PSA), a soluble form of picryl chloride, and can be transferred by the 4^{th} day after sensitization (22). The tolerance is antigen specific, T cell dependent, and does not result in decreased DNA synthesis or cell proliferation but seems to act on the efferent limb of sensitization by inhibiting the expression of immune lymph node cells.

More recently the same investigators have identified another suppressor T cell population in the picryl chloride system (80). These T_s are generated by injecting 30×10^6 spleen cells from mice immunized with picryl chloride painting, into normal mice which are then immunized 5 days later with picryl chloride. The injected T_s were found to suppress <u>in vivo</u> synthesis in the normal mice after 4 days. This T_s differs from the T_s for contact sensitivity induced by the injection of picryl sulphonic acid (22) in that it did not affect contact sensitivity, its appearance was prevented by cyclophosphamide and adult thymectomy had no effect while the reverse was true for the T_s for contact sensitivity (80).

Greene, et al have described a different technique of eliciting delayed type contact sensitivity reactions in mice in the picryl chloride system, that of subcutaneous immunization of syngeneic 3 x 10^7 TNP-modified cell or membranes followed by challenging with picryl chloride painted on the skin (82). This TNP-induced contact sensitivity response was found to be T cell dependent, could be transferred and was subject to suppression by 5 x 10^7 spleen cells from animals which had been tolerized with i.v. injection of TNP cell membrane corresponding to 10^8 cells (82). The kinetics of suppression showed that i.v. treatment 5 days before subcutaneous sensitization produced maximal suppression (82).

Scott and Long were able to demonstrate B cell tolerance in rats that were treated intravenously with 1 x 10^7 TNP-modified spleen cells or lymph nodes as evidenced by decreased number of plaque-forming cells to TNP-protein conjugates (83). Tolerance was achievable with as few as 10^5 to 10^6 TNP spleen cells, required a latent period of 3-4 days to develop, lasted 4 weeks, and waned by 6 weeks (83). Injection of TNP-modified spleen cells also resulted in suppression of hapten delayed hypersensitivity (83). Induction of tolerance required cyclophosphamide sensitive T cells and H-2 identity was not required (84) as contrasted with the results of Miller, et al. (78).

Ptak and Rozycka found that i.v. injections of TNP bound syngeneic erythrocytes or thymocytes tolerized mice to picryl

chloride, abrogating contact sensitivity while leaving humoral anti-TNP responses intact (85). However, i.v. injections of TNP-bound isologous IgG abrogated anti-TNP responses with no effect on contact sensitivity to picryl chloride (85). TNP-bound macrophages result in suppression of both humoral and cell-mediated responses (85). Thus the "split unresponsive state" induced by TNP may be mediated by two separate mechanisms which are triggered by different presentation modes.

The DNFB contact sensitivity system of Claman, Moorhead and colleagues is characterized by sensitization of mice with direct painting of the DNFB on the skin, and challenge painting 5 days later, followed by quantitation of contact sensitivity by measurement of ear swelling 24 hours later (73). Tolerance is induced 7 days before sensitization by i.v. injection of $DNBSO_3$ (a soluble form of DNFB, DNFB bound to either intact cells (lymphoid or erythrocytes) or cell ghosts (75). It was found that the stronger the binding between the DNFB and cell membranes, the more potent the tolerance (75). Moreover, intact cells were not required, merely membrane bound DNFB (75). Tolerance could be adoptively transferred to normal recipients from tolerant donors and this transfer is T cell dependent (76). The tolerance induced or transferred is exquisitely specific for DNFB (73). The kinetics of this system are such that the induction of tolerance occurs within 24 hours, yet the ability to transfer tolerance required a period of 4-7 days to develop after i.v. induction and was lost 14 days after which the

animal remained tolerant (77). The authors inferred that there are two mechanisms of tolerance to DNFB: 1) a finite period of antigen specific suppressor T cell activity which requires several days to develop which is responsible for the transfer of tolerance and 2) the inhibition of DNFB specific T cell clones from receptor blockade by hapten-modified self membranes (77). The generation of T_s in this system has been shown to be H-2 restricted (78,79). Syninduced T_s suppress the efferent limb of contact sensitivity by blocking the expression of immune lymph node cells (25) and alloinduced T_s suppress the early afferent limb of sensitization by blocking cell proliferation (26).

Moody, et al found that the i.v. injection of 10^6 TNP coupled syngeneic mouse erythrocytes induced suppression of the anti-DNP response to DNP-BGG (87). Minimal suppression occurred 7-10 days before immunization and waned by the 17^{th} day. Suppression was found to be T cell dependent and hapten specific but not exquisitely so, since TNP-induced suppression depressed the response by a DNP immunogen.

Suppression of delayed hypersensitivity to azobenzene arsonate (ABA) in mice has recently been demonstrated (86). The suppression was induced by the i.v. injection of 5×10^7 ABA-modified spleen cells followed by splenectomy 7 days later and transfer of the spleen cells into a recipient that was simultaneously immunized subcutaneously with ABA-coupled spleen cells. The suppression of DTH was found to be antigen-specific and T cell dependent (86).

RATIONALE AND OBJECTIVES OF THESIS

The various models of hapten-specific suppression described above have been very useful in elucidating the mechanism of suppression, but are all characterized by haptens which are irrelevant to the immune response of naturally occurring antigens. The model of suppression to be described in this thesis utilizes nucleosides as the hapten. Nucleosides, i.e. ribose (or deoxyribose) conjugates of the purine (guanine and adenine) and pyrimidine (thymine and cytosine) bases of nucleic acids were chosen because previous work by Borel and Stollar (116) utilizing nucleoside coupled to isologous protein carriers demonstrated mucleoside specific tolerance. The technique of coupling determinants larger than nucleosides such as oligonucleotides and nucleic acids is currently being perfected, and will be applied to the model described herefor nucleosides. It was thought that by using the techniques of coupling nucleosides to protein devised in Borel's nucleoside tolerance model to similarly bind nucleosides to lymphoid cells, it might be possible to generate a hapten-specific model of suppression for nucleosides analogous to the models reviewed above. The results of this thesis show that such a model of nucleoside suppression can indeed be generated.

The objectives of this thesis are:

(1) to demonstrate that nucleoside-coupled lymphoid cells can induce T cell mediated suppression to anti-nucleoside antibody production <u>in vivo</u> and <u>in vitro</u>,

(2) to characterize the parameters of this model, namely: dosage requirements, kinetics and the specificity of nucleoside suppression,

(3) to assess the role of the cell carrier in the efficacy of this suppression and the possibility of an H-2 requirement for suppression,

(4) to ascertain whether there are strain differences in suppression and whether or not a strain resistant to suppression exists,

(5) and finally, to determine if suppression to nucleosides can be induced in NZB and BWF_1 mice.

MATERIALS AND METHODS

ANIMALS

C57BL/6J, (C57/6J X DBA/2)F_1 (hereafter referred to as BDF_1), DBA/2, BALB/c, SJL/J males were purchased from Jackson Laboratories, Bar Harbor, Maine. NZB males were bred from a parental stock from the National Institute of Health, Bethesda, MD. Animals were fed laboratory chow. All animals entered experiments at ages 6 to 8 weeks unless otherwise specified.

PREPARATION OF IMMUNOGENS AND TOLEROGENS

HAPTENS
(Nucleosides)

Guanosine, adenosine and cytidine were purchased from Sigma, St. Louis, Mo. Thymine riboside was purchased from Calbiochem, LaJolla, CA.

PROTEIN CARRIERS

Keyhole limpet hemocyanin (KLH) was obtained from Pacific Bio Marine Supply Co., Venice, CA.

IgG2a was isolated from the serum of plamacytoma-bearing BALB/c mice by starch block electrophoresis.

Nucleosides were conjugated to BALB/c IgG2a (RPC_5) myeloma protein as tolerogen or to keyhole limpet hemocyanin as immunogen by the procedure of Erlanger and Beiser (89). A mixture containing approximately 80 umole of each nucleoside was oxidized with 10 ml of 0.1 M sodium periodate and added to 150 mg of IgG2a (1 umole). After reduction with sodium borohydride and extensive dialysis of the product, the conjugate

was assayed for protein by a microbiuret method and for nucleoside content by UV spectroscopy. The spectrum was corrected for protein content and a millimolar extinction coefficient of 10 was used as average for the four nucleosides. The immunogen preparation was $(AGCT)_{115-125}$-KLH and G_{100}-KLH (the subscript numbers indicate the total molar ratio of hapten substitution on the carrier assuming 800,000 as the molecular weight of KLH). Tolerogen preparation was G_{25}-IgG2a.

PREPARATION OF CELL SUSPENSIONS

SPLEEN CELLS (SC)

Animals were sacrificed by cervical dislocation and the spleens were removed and homogenized together in a 15 ml glass tissue grinder (Wheaton) in 15 ml of Minimal Eagle's Medium (MEM), (Microbiological Associates, Walkersville, MA), washed by centrifugation at 1000 RPM for 15 minutes three times in 50 ml MEM and once in 50 ml of a 50% solution of MEM and 0.15 M $NaHCO_3$ at pH 8.0. The cells were resuspended in 2 ml of the 50% MEM-$NaHCO_3$ solution and the cell concentration determined in a hemocytometer.

THYMUS CELLS (TC)

The thymus gland was carefully dissected from each mouse and gently teased in MEM. Thymus cells were then washed three times in MEM and once in 50 ml of the 50% MEM-HCO_3 solution as with the spleen cells.

SHEEP RED BLOOD CELLS (SRBC)

SRBC's were purchased from Colorado Serum Co., Lab, Denver, Colorado and stored at $4^{o}C$. Prior to use in hemolytic plaque assay or i.v. injection, SRBC's were washed three times in 0.15 M $NaHCO_3$.

MOUSE RED BLOOD CELLS (MRBC)

C57BL/6 males were bled under a heat lamp; blood was added to an equal volume of Alsever's solution and stored at $4^{o}C$. When ready for use, MRBC were washed in 0.15 M $NaHCO_3$ three times. Cells then counted by hemocytometer and appropriate adjustments made in concentration.

HORSE RED BLOOD CELLS (HRBC)

HRBC's were purchased from Colorado Serum Co. Lab, Denver, Colorado, and stored at $4^{o}C$. Prior to use, HRBC's were washed three times in 0.15 M $NaHCO_3$, counted by hemocytometer and adjusted in concentration.

NUCLEOSIDE MODIFICATION OF LYMPHOID CELLS

For every 10^9 cells, 10 mg of guanosine, or 5 mg each of guanosine (G), adenosine (A), thymine riboside (T), and cytosine (C) for a total of 20 mg of tetranucleoside (AGCT), were used. Nucleoside(s) was covalently linked to cells by the method of Stollar & Borel (90) but modified to prevent lymphoid cell lysis.

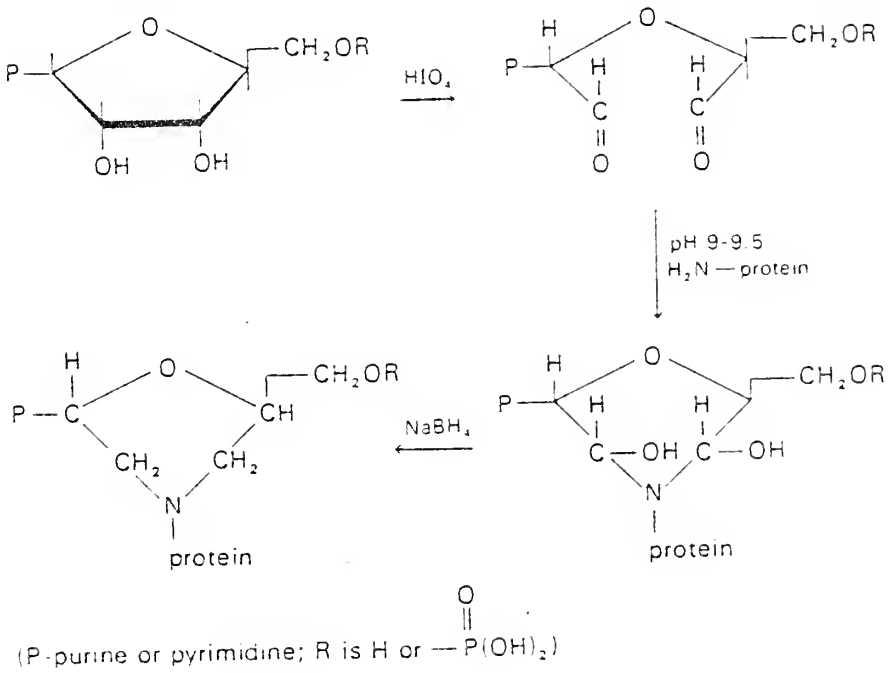
10 mg of guanosine were suspended in 3 ml of 0.15 M $NaHCO_3$ and then oxidized with 1.5 ml of 0.1 M sodium periodate in saline for 20 minutes at room temperature. The reaction was stopped with 15 ul of ethylene glycol which breaks down excess periodate

(89). The cell suspension was added dropwise to the oxidized nucleoside solution and the mixture was gently stirred at room temperature. The binding was stopped after 15 minutes with 100 mg t-butylamine borane in 5 ml 0.15 M $NaHCO_3$. After 3 minutes at room temperature, the reaction tube (50 ml Falcon) was filled to 50 ml with MEM and centrifuged for 15 minutes at 1000 RPM. The nucleoside-conjugated cells were then washed with MEM 3 more times after which appropriate adjustments in cell concentration were made. Cells were then ready for intravenous injection via tail vein. Sham modified cells were prepared in an identical manner except for the addition of 1.5 ml of sodium periodate without guanosine. Subsequent treatment with ethylene glycol, t-butylamine borane and washings were unchanged. Groups of 5 mice were used.

Nucleoside modification of MRBC, SRBC, and HRBC were performed by the procedure of Stollar and Borel (90), in essentially the same manner as above, except washes were with 0.15 M $NaHCO_3$ buffer without added MEM, and centrifugations were performed at 2000 RPM.

After the basic system for suppression was established (see RESULTS section) with an optimal cell dose of 80 x 10^6 i.v. on day 0 followed by immunization with 0.2 mg nucleoside-KLH in Complete Freund's Adjuvant (Difco, Detroit, Mich.) and hemolytic plaque assay on day 10, the method for binding nucleoside to cells was reevaluated.

The method described above is based on the method of Erlanger and Beiser used to conjugate ribosides to protein (89). The reaction scheme is as follows:



(P-purine or pyrimidine; R is H or $-\overset{O}{\overset{\|}{P}}(OH)_2$)

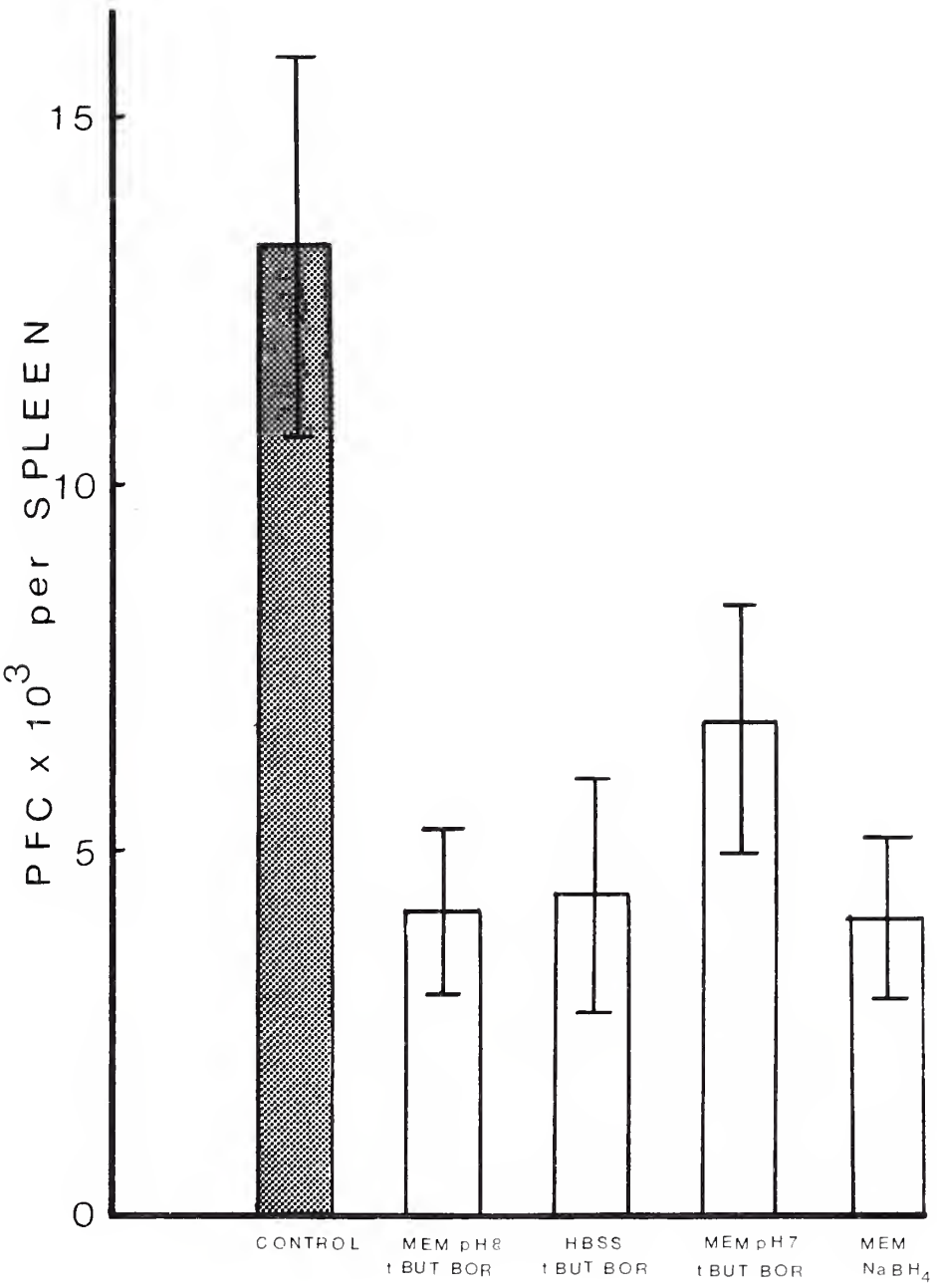
The reaction involves oxidizing the ribose ring with periodate to give a dialdehyde that couples to free amino groups. The resultant bond is stabilized by sodium borohydride, a reducing agent. (91) Stollar and Borel modified this procedure for linking nucleoside to sheep erythrocytes for use as target cells in the hemolytic plaque assay (see below). The modifications consisted of the use of 0.15 M bicarbonate to maintain ionic strength to prevent lysis and provide a suitable pH for Schiff base formation, and the use of t-butylamine borane complex in place of sodium borohydride, to prevent membrane damage during reduction and to decrease reduction time (90). To bind nucleoside to lymphocyte membranes, further modifications were

necessary to prevent lymphoid lysis--namely, to lower the pH from 9.5 to 8.0 by adding an equal volume of MEM at physiological pH to the 0.15 M bicarbonate buffer.

Variations were tried on the above method to attempt to evaluate the binding and improve it if possible. In addition to the 80 x 10^6 cells bound to guanosine in the usual manner (MEM at pH 8/ t-butylamine borane (Aldrich Chemical Co., Inc., Milwaukee, WI)), Hank's balanced salts solution (HBSS) (Microbiological Associates, Walkersville, MA) was used in place of MEM in an attempt to assess the effect of lowering the number of free amino groups on binding and reduction by t-butylamine borane. Another group consisted of using MEM at physiological pH to optimize conditions for cell viability. The original reducing agent sodium borohydride was used in place of t-butylamine borane in the final group.

The results shown in Fig. 1. All groups suppressed significantly. The three groups exhibiting the most suppression were the usual method, HBSS replacing MEM and $NaBH_4$ replacing t-butylamine borane. Cell viabilities were 80 to 85% for all groups except for the group using $NaBH_4$ which had 30% viability. Therefore, it appears that viable cells are not required for generation of suppression in this system, confirming Claman and Miller's results (75). Since the original method proved to be as effective as the variations, no change was made in the basic procedures of this project.

FIG. 1 The effect of varying conditions for the binding of nucleoside on suppression of the immune response to G-KLH. 80 x 10^6 syngeneic spleen cells coupled to guanosine by 5 different conditions of binding, were injected i.v. into groups of 5 BDF_1 mice with a group of untreated mice as controls. All mice were immunized on day 5 with 0.2 mg G-KLH in CFA i.p. Individual spleen suspensions were made from each mouse on day 20 and assayed for PFC against G-SRBC targets. Each bar represents the geometric mean of the group $\pm$ s.e.


15
10
5
0
PFC x 10^3 per SPLEEN
CONTROL
MEM pH8 t BUT BOR
HBSS t BUT BOR
MEM pH7 t BUT BOR
MEM $NaBH_4$

INDUCTION OF SUPPRESSION

Animals were preheated under a heat lamp for vasadilatation. Intravenous injections of cell suspensions were made with a 23 gauge needle via tail vein.

IMMUNIZATIONS

0.2 mg of G-KLH or AGCT-KLH in an equal volume of Complete Freund's Adjuvant was injected per animal intraperitoneally. (94)

IN VITRO CULTURES

Spleen cell suspensions from either untreated C57BL/6 mice or C57BL/6 mice injected intravenously with 80 x 10^6 guanosine-coupled spleen cells 7 days before, were cultured in Marbrook-Diener tissue culture conditions *in vitro* (92). Spleen cells were challenged *in vitro* with guanosine$_{58}$-AECM-Ficoll (gift of Dr. B. David Stollar) or sheep red cells, or without antigen for evaluation of background. Four days later, the cells were harvested and assayed by PFC against either G-SRBC or SRBC as target.

HEMOLYTIC PLAQUE ASSAY

The hemolytic plaque assay of Jerne (93) as modified by Stollar and Borel (94) was used to assay the immune response to the immunogen nucleoside-coupled KLH. This is measured in terms of number of plaque forming cells (PFC) making anti-nucleoside antibodies directed against nucleoside coated target cells.

Nucleoside-coupled sheep erythrocyte targets were prepared by oxidizing nucleoside or tetranucleoside in the same manner as described above for binding to lymphocytes. SRBCs were washed twice in 0.15 M bicarbonate buffer and 0.5 ml packed cells was suspended in 2.0 ml 0.15 M bicarbonate buffer to make a 20% suspension. The cells were then added to the oxidized nucleosides, binding for 15 minutes at room temperature, reduced with 100 mg t-butylamine borane and washed three times with 0.15 M bicarbonate.

Animals were sacrificed and individual spleen suspensions were made by expressing the tissue through surgical tantalum gauze into 5 ml of MEM. 0.05 ml of the nucleoside-coated SRBCs and 40 ul of the spleen suspension were added to 1 ml of 1% Agar (Difco Lab., Detroit, Mich.) in MEM, and the mixture was poured in 60 x 15 mm tissue culture dishes (Falcon). The plates were incubated at 37° C. for one hour before 1 ml/ plate of 1:30 diluted guinea pig complement (Cappel Laboratories, Downingtown, PA) was added. The plates were read after an additional 45 minutes incubation at 37° C. for direct plaque forming cells (PFC). Indirect plaque forming cells were read after incubating the plates with 1 ml/plate of 1:200 diluted rabbit anti-IgG for an additional hour after reading direct PFC. To obtain indirect PFC, direct PFC were subtracted from total PFC read after incubating with anti-IgG. No indirect PFCs were observed in this system so they were not routinely done.

STATISTICAL ANALYSIS

Statistical analysis was done according to Student's t test for significance of difference in PFC numbers between groups. The geometric means $\pm$ standard errors were expressed. $p < 0.05$ was judged significant.

RESULTS

BASIC SYSTEM

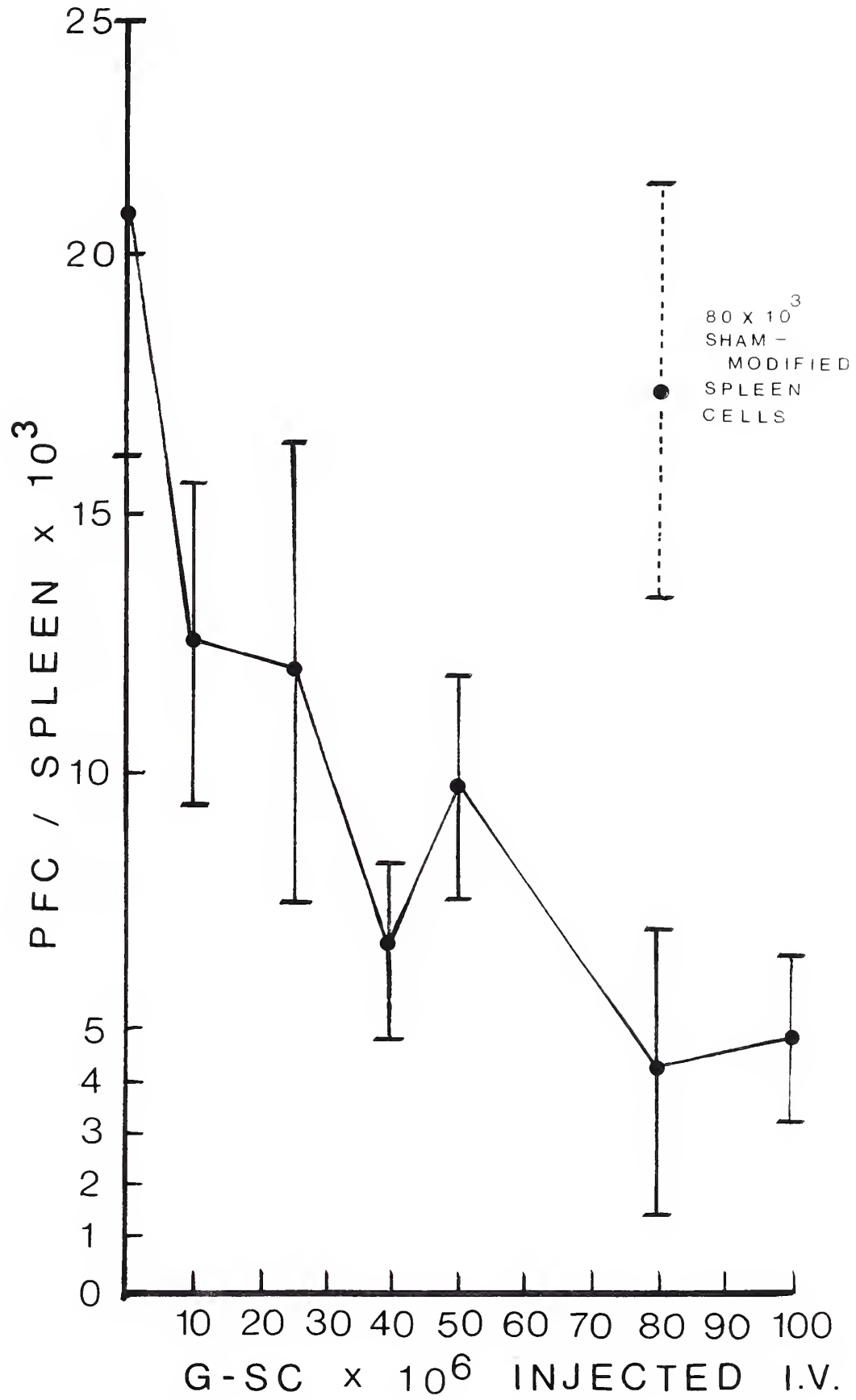
SUPPRESSION BY GUANOSINE-COUPLED SPLEEN CELLS

Preliminary experiments were done to establish whether or not nucleoside-coupled spleen cells could induce suppression of the animal's immune response to nucleoside-coupled KLH, a T dependent antigen. Guanosine was chosen to be the nucleoside in these initial experiments because this nucleoside has been shown to be immunodominant in the immune response to all four nucleosides of DNA (94). Groups of 5C57BL/6 male mice were injected intravenously with varying doses of G-coupled spleen cells (G-SC). Control groups consisted of untreated mice or mice injected with sham-modified spleen cells. Five days later, all animals were immunized with intraperitoneal injections of 0.2 mg guanosine-KLH in complete Freund's adjuvant. Direct anti-guanosine plaque forming cells were assayed from individual spleen suspensions using G-SRBC targets. Suppression of the anti-guanosine PFC response was found to be dose-dependent (Fig. 2). The optimal dose was 80×10^6 G-coupled spleen cells; increasing the dose had no additional effect on suppression. 80×10^6 sham-modified spleen cells did not suppress the anti-G immune response at all.

KINETICS OF SUPPRESSION

The time course of suppression was then determined by changing the length of time between the i.v. injection of G-coupled spleen cells and the i.p. immunizing challenge with G-KLH. Using the optimal dose of 80×10^6 guanosine-coupled

Fig. 2 The dose response kinetics of suppression of the immune response to guanosine-KLH induced by intravenous injection of guanosine-modified spleen cells. Groups of five C57Bl/6 mice were injected i.v. with 80 x 10^6 G-coupled syngeneic spleen cells. Five days later, all mice together with a control group of five untreated mice, were immunized i.p. with 0.2 mg G-KLH in CFA. PFC against G-SRBC targets were assayed on day 10. Each point represents the geometric mean of PFC per spleen ± s.e. of each group of 5 mice. Statistical analysis by student's T Test.

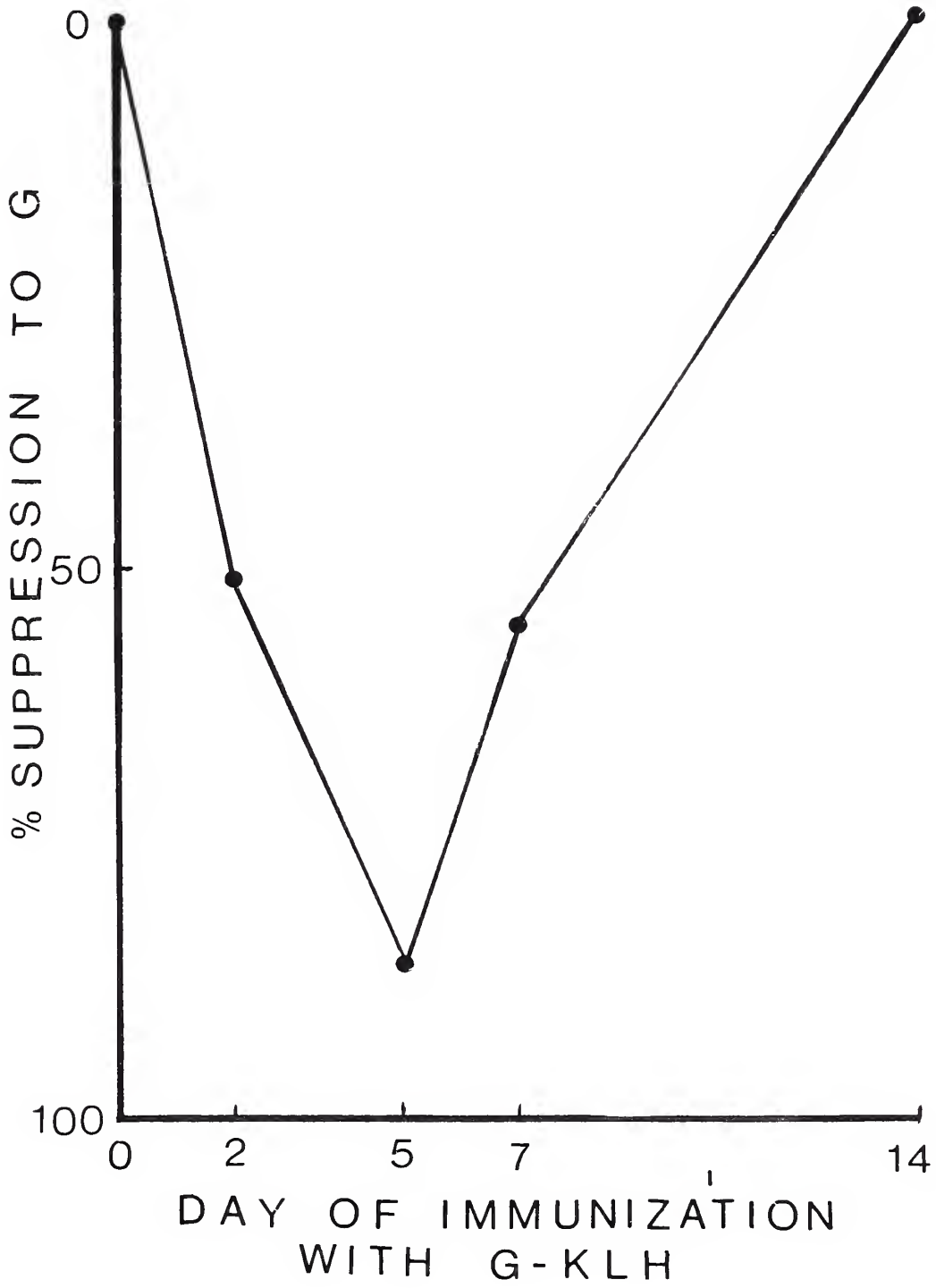

25
20
15
10
5
4
3
2
1
0
PFC / SPLEEN x 10^3
80 X 10^3
SHAM –
MODIFIED
SPLEEN
CELLS
10 20 30 40 50 60 70 80 90 100
G-SC x 10^6 INJECTED I.V.

spleen cells, the time course of suppression was found to have a short latent period 24 to 48 hours after the i.v. injection, to reach a maximum level of suppression when immunization was done 5 days after i.v. treatment, and to be completely gone if immunization was done 2 weeks later. (Fig. 3).

IN VITRO SUPPRESSION

The short latent period and the transience of suppression is characteristic of many other systems (77, 83, 87), and is suggestive that this system like the others, involves T cell mediated suppression. To substantiate this, spleen cell suspensions from C57BL/6 mice were injected i.v. with 80×10^6 G-coupled spleen cells, were cultured *in vitro* 7 days later and challenged with a T independent antigen G_{58}-AECM-Ficoll, with SRBC or no antigen. Four days later, the cultures were harvested and assayed for direct PFC to guanosine and SRBC. The results in Table 1 show that the spleen cells of animals treated with G-spleen cells could suppress the B cell response to a T independent antigen, G-Ficoll. The suppression is antigen specific as shown by the SRBC response, which was unchanged.

Fig. 3 Kinetics of the suppression of the immune response to guanosine-KLH. Groups of five C57Bl/6 mice were injected i.v. with 80 x 10^6 G-coupled spleen cells on day 0. Immunization of all mice together with a control group of five untreated mice, were immunized i.p. with 0.2 mg G-KLH in CFA on varying days after i.v. treatment. PFC against G-SRBC were assayed five days later. Each point represents % suppression of experimental group from immune control group.


0
50
100
% SUPPRESSION TO G
0
2
5
7
14
DAY OF IMMUNIZATION
WITH G-KLH

SPECIFIC SUPPRESSION OF GUANOSINE IMMUNE RESPONSE
TO A T-INDEPENDENT ANTIGEN *IN VITRO*

TREATMENT OF MICE BEFORE IMMUNIZATION *IN VITRO*	antigen target	G-Ficoll G-SRBC	p	SRBC SRBC
NONE		550±176		5688±1216
80×10^6 G-SC i.v.		56±129	.05	5557±1004

TABLE 1. Spleen cell suspensions from either 80×10^6 G-SC i.v. treated or untreated C57BL/6 mice cultured 7 days later in Marbrook-Diener tissue culture conditions and challenged *in vitro* with either Guanosine_{58} AECM-Ficoll or SRBC or without antigen. Cultures harvested after 4 days and assayed for PFC against G-SRBC or SRBC targets. Each figure represents the mean PFC of four cultures ± s.s. minus background. PFC background to SRBC was 12±63 and background to G-SRBC was 14±99. Statistical analysis was done according to Student's t test.

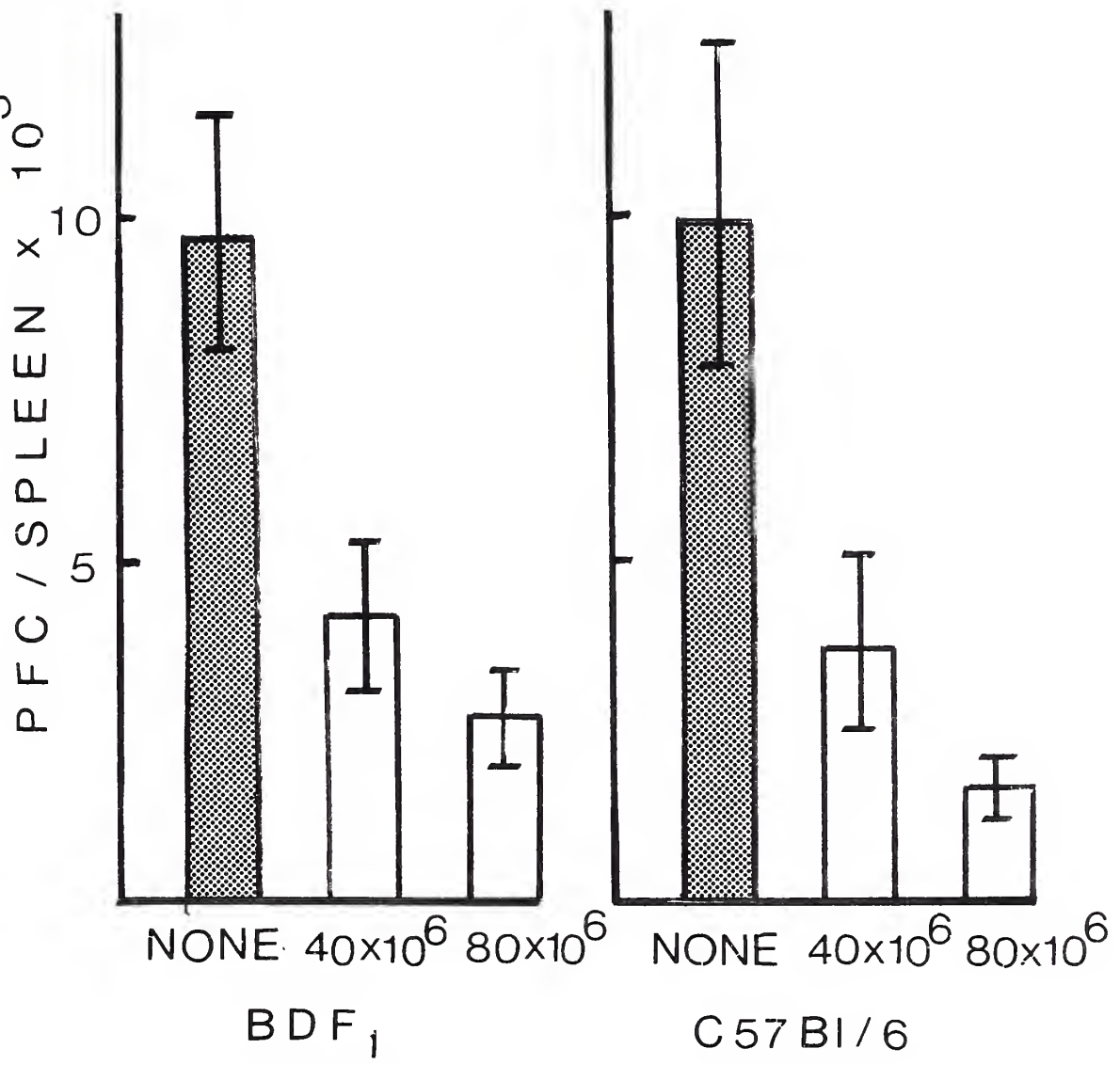
CAN TETRANUCLEOSIDE BE SUPPRESSED?

The tetranucleoside (AGCT) was coupled to spleen cells of either C57BL/6 or BDF_1 mice and 80 x 10^6 or 40 x 10^6 AGCT-coupled spleen cells were injected i.v. into syngeneic recipients. Five days later, these mice together with a control group of untreated mice of the same strain, were immunized with tetranucleoside coupled to KLH (AGCT-KLH). The response to AGCT-KLH was assayed by direct PFC to AGCT-SRBC targets. The results (Fig. 4) show almost identical suppression by the two strains to both doses of AGCT-SC.

SPECIFICITY OF SUPPRESSION

The specificity of the nucleoside tolerance system of Borel is exquisite; when the animal is tolerized to guanosine with guanosine-IgG2a and subsequently immunized with AGCT-KLH, an antibody response is produced to any nucleoside not conjugated to the IgG carrier (90). To assess the specificity of our nucleoside suppression systems, groups of five C57BL/6 mice were suppressed with 80 x 10^6 guanosine-SC i.v. and challenged with AGCT-KLH on day 5. To ascertain if the animals were responding with just anti-G or anti-AGCT or other antibodies, a panel of 5 differently coupled SRBC targets was provided: AGCT-SRBC, A-SRBC, G-SRBC, C-SRBC and T-SRBC, for assay on day 10. For comparison, a group was tolerized to G with G-IgG2a; control groups consisted of mice that received 80 x 10^6 sham-modified SC and an untreated group.

Fig. 4 Comparative suppression of the immune response to AGCT-KLH induced by syngeneic AGCT-KLH induced by syngeneic AGCT-Modified spleen cells in C57Bl/6 and BDF mice. Groups of five mice of each strain were treated i.v. with 30×10^6 and 40×10^6 syngeneic AGCT-modified spleen cells. Immunization with AGCT-KLH and PFC against AGCT-SRBC targets were performed similarly as described in Fig. 2.


PFC / SPLEEN x 10^3
10
5
NONE
40x10^6
80x10^6
NONE
40x10^6
80x10^6
BDF1
C57Bl/6

AGCT · SC i.v.

NUCLEOSIDE SPECIFICITY OF SUPPRESSION IN VIVO
PFC/SPLEEN(±SE)

GROUPS	TREATMENT 5 DAYS BEFORE IMMUNIZATION	AGCT-SRBC	p	A-SRBC	p	G-SRBC	p	C-SRBC	p	T-SRBC	p
I	NONE	21,824±8382		6428±2160		18,808±8908		10,108±3744		8502±3309	
II	$80x10^6$ SHAM-Spl.	20,328±4274		6009±1514		17,178±4657		7757±1892		8555±2497	
III	G-Igd2a	11,454±2250	0.05	4569±1172	0.2	4821±2893	0.01	9443±2306	0.5	7853±2968	0.5
IV	$80x10^6$ G-Spl.	11,501±2849	0.05	2868±713	0.005	5789±1797	0.025	4484±790	0.005	6844±1482	0.2

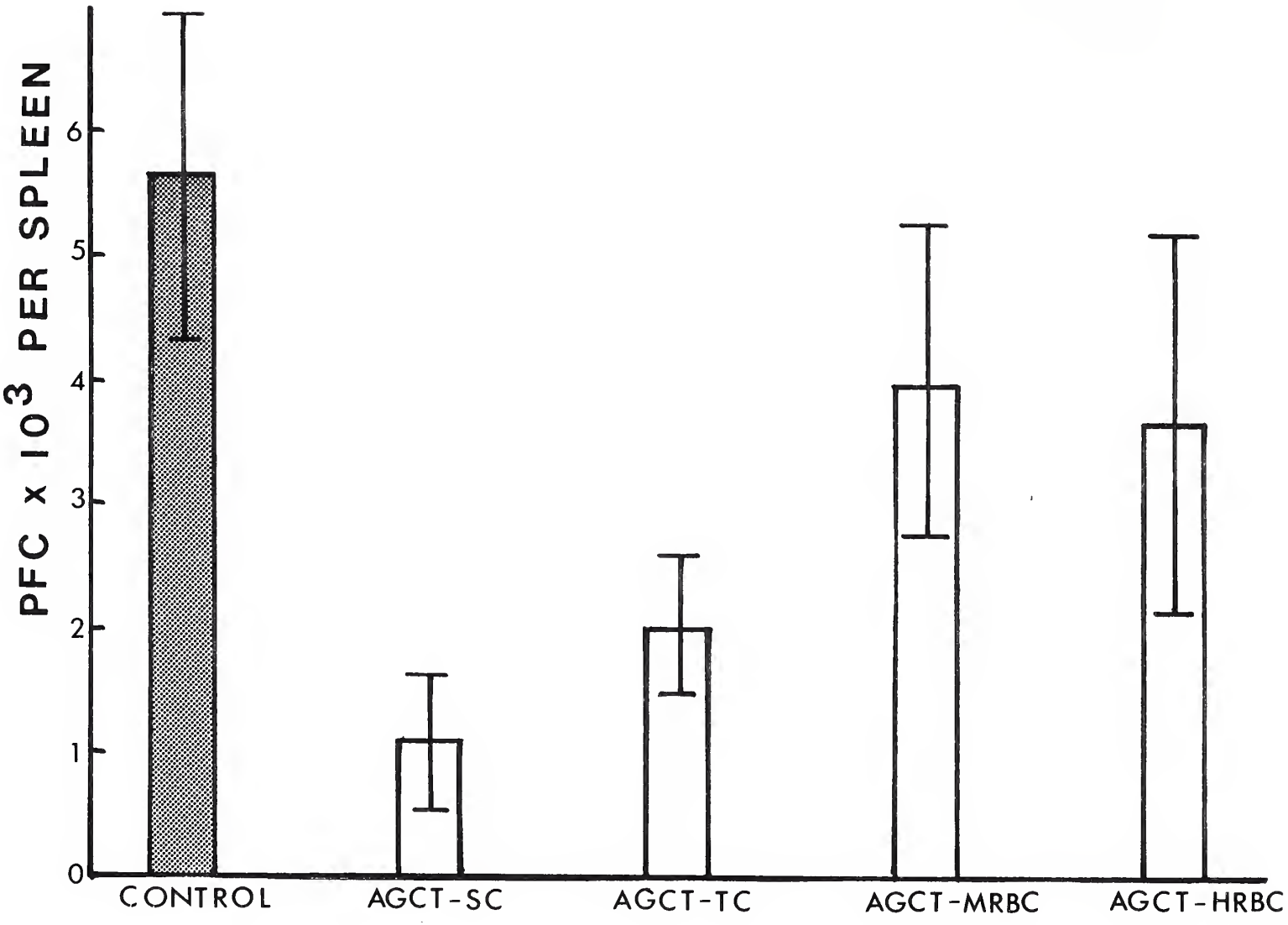
TABLE 2. Groups of C57BL/6 mice were treated i.v. 80 x 10^6 G-SC. Controls consisted of an untreated group, a group receiving 80 x 10^6 sham-modified SC, and a group made tolerant to G with 0.2 mg G-IgG2a. All mice were immunized i.p. 5 days later with 0.2 mg AGCT-KLH in complete Freund's Adjuvant Animals were sacrificed on day 10 and individual spleen cell suspensions were made and assayed for direct PFC against targets coated with AGCT as well as targets coated with A, G, C, T individually. Values are geometric means of PFC per spleen $\pm$ s.e.

The immune response of these G-SC treated animals to SRBC was also examined. The results show that treatment with G-SC affects not only the immune response to the hapten on the modified lymphoid cell i.e. guanosine, but also the immune responses to adenosine and cytosine as well. In contrast, tolerance induced by G-IgG2a was specific for guanosine. Thus, the specificity of suppression induced by G-coupled SC is broader than the tolerance induced by G-IgG2a. The suppression was specific for nucleosides, however, as the immune response to SRBC, an unrelated antigen, was unaffected in vivo. (PFC to SRBC = 158,700 ± 30,200 in G-SC treated mice five days before challenging with 0.1 ml of 20% SRBC suspension, and PFC = 158,600 ± 21,500 in untreated mice similarly challenged.)

ROLE OF THE CELL CARRIER

Thus far in our system, the spleen cell seems to be a very effective cellular carrier for hapten-specific tolerance. The next experiment varied the cell carrier in an effort to discover whether any other cell type might serve as an optimal carrier for nucleosides. The results are shown in Fig. 5. Syngeneic splenocytes were the most effective carriers for suppression; thymus cells induced significant suppression but only half as well as splenocytes. Syngeneic and xenogeneic erythrocytes were ineffective carriers of nucleoside suppression.

FIG. 5 The role of the cell carrier in suppression of the immune response to AGCT-KLH. 80 x 10^6 cells of different types were coupled to AGCT and injected i.v. into groups of 5 C57BL/6 mice. All mice together with a control group of untreated mice were immunized 5 days later with 0.2 mg AGCT-KLH in CFA i.p. PFC against AGCT-SRBC targets were assayed on day 10. Each bar represents the geometric mean of each group $\pm$ s.e.

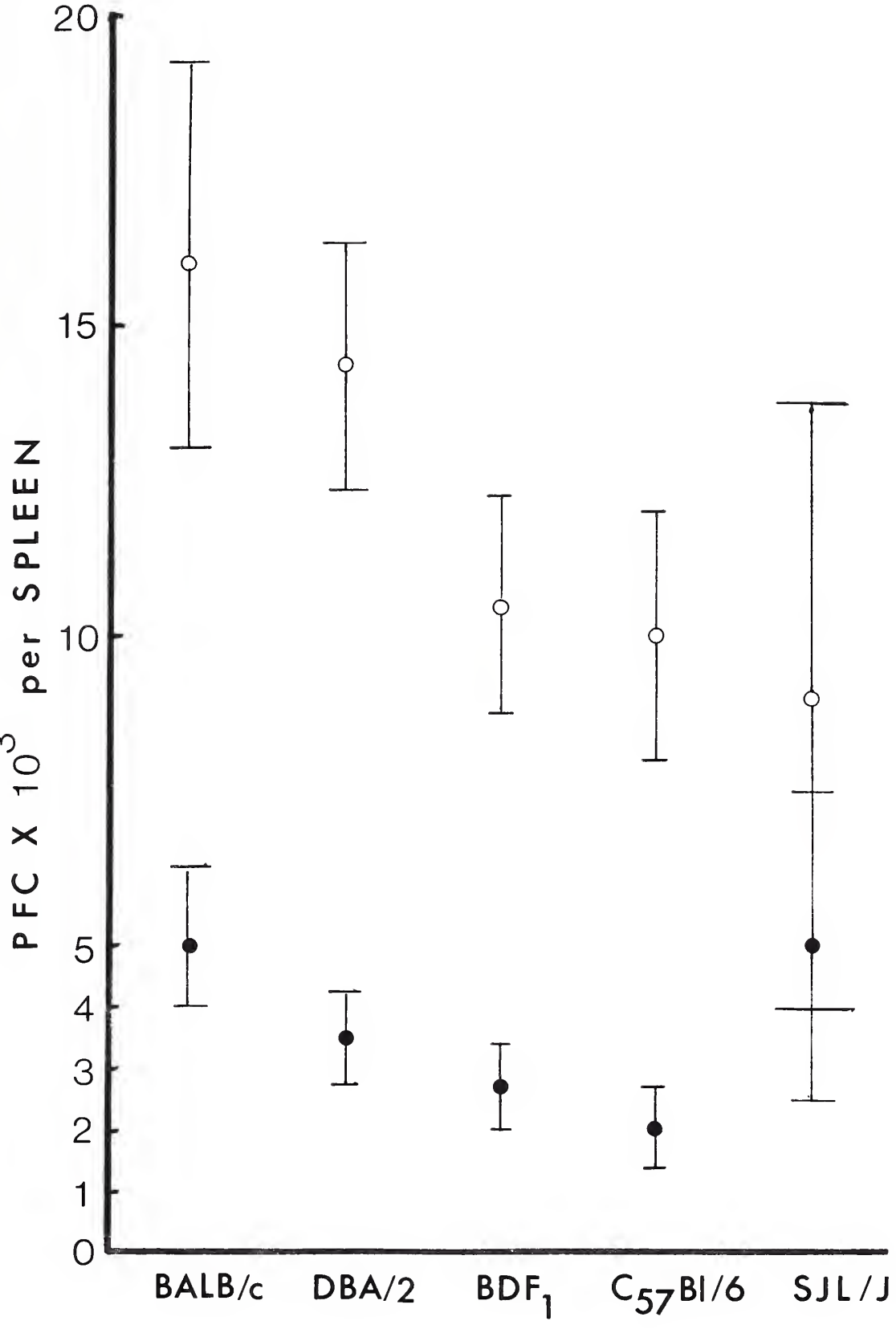

PFC x 10^3 PER SPLEEN
0
1
2
3
4
5
6
CONTROL
AGCT-SC
AGCT-TC
AGCT-MRBC
AGCT-HRBC

STRAIN DIFFERENCES IN NUCLEOSIDE SUPPRESSION

All the data presented have been in the C57BL/6 and the BDF_1 strains. Interestingly, the comparative data between the parent and the F_1 with regard to AGCT-directed suppression were very similar. (Fig. 4) The next question asked concerned whether there existed any strain differences in the generation of suppression and whether there existed any strains which were resistant to suppression with nucleosides. The DBA/2 (the other parental strain of the BDF_1), the SJL/J and the BALB/c were tested, using the dose of 80×10^6 spleen cells coupled to AGCT and administered i.v. into syngeneic recipients. The results are presented in Fig. 6. All strains could be suppressed except for the SJL/J which did not exhibit significant suppression.

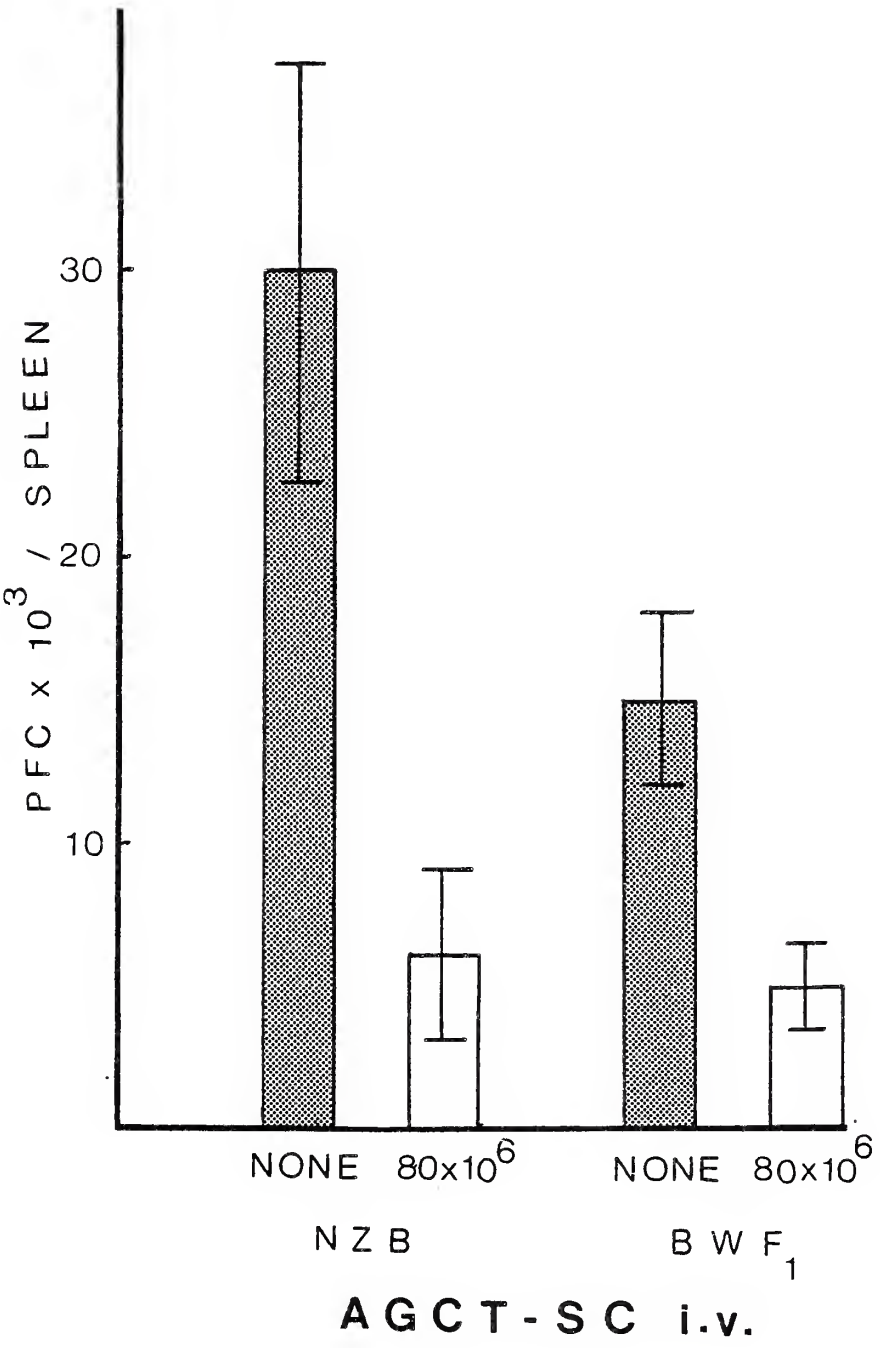
The final experiment to be presented represents the natural endpoint for the techniques generated by this system--namely, the investigation of T cell suppression in the New Zealand mice: the New Zealand Black (NZB), the New Zealand White (NZW) and the (NZB X NZW)F_1 or the BWF_1. This system would enable the investigation of the current notion that the pathogenesis of autoimmunity in the NZB/W mice is due to loss of suppressor cell activity with age allowing forbidden clones of lymphocytes with autoantigen specificity to develop (58,111).

FIG. 6 Strain differences in the suppression of the immune response to AGCT-KLH, induced by syngeneic AGCT-coupled spleen cells. $\overset{\circ}{\mathrm{I}}$ represents immune controls. $\overset{\bullet}{\mathrm{I}}$ represents suppressed response. 80×10^6 AGCT-coupled syngeneic spleen cells were injected i.v. into groups of five mice. Five days later, all mice were immunized with 0.2 mg AGCT-KLH in CFA i.p. PFC against AGCT-SRBC targets were assayed on day 10. Each bar represents the geometric mean of each group $\pm$ s.e.


20
15
10
5
4
3
2
1
0
PFC X 10^3 per SPLEEN
BALB/c
DBA/2
BDF_1
$C_{57}Bl/6$
SJL/J

Relatively young (8 - 12 weeks) male NZB mice and 6 week old male BWF, micewere injected i.v. with 80 x 10^6 AGCT-coupled syngeneic spleen cells and immunized on day 5 with 0.2 mg AGCT-KLH per usual. The result is shown in Fig. 7. A high degree of suppression in both strains was observed.

FIG. 7 The suppression of the immune response to AGCT-KLH induced by syngeneic AGCT-coupled spleen cells in 8 to 10 week old NZB male mice and 6 week old BWF_1 male mice.


PFC x 10^3 / SPLEEN
30
20
10
NONE
$80x10^6$
NONE
$80x10^6$
N Z B
B W F_1
AGCT-SC i.v.

DISCUSSION

Suppression induced by a variety of haptens presented on cells has been extensively investigated as reviewed above. In this thesis is presented a new model of suppression directed against the immune response to nucleosides, naturally occurring haptens which are components of nucleic acids. Suppression to nucleosides is cell dose dependent, requires a latent period to develop and has transient kinetics. Suppression was induced against both a T dependent antigen *in vivo* and a T independent antigen *in vitro*; this together with the kinetic profile is strongly suggestive of T cell mediated suppression. Anti-nucleoside suppression is antigen-specific but lacks the exquisite specificity of carrier-induced tolerance. Except for SJL/J mice, anti-nucleoside suppression was demonstrated in all strains of mice tested including the NZB and BWF_1 mice. Preliminary data show that the spleen cell is the most effective cell carrier for suppression and suggests that there is an H-2 requirement for anti-nucleoside suppression.

In this model of anti-nucleoside suppression, nucleosides are coupled directly to cells by covalent bonding to membrane proteins. This coupling is stable after reduction. There is no secondary binding of the nucleoside *in vivo* once this coupling has occurred. Whether the resultant nucleoside-membrane complex is catabolized and/or pinocytosed is not known.

After covalent conjugation, the nucleoside-modified cells were injected intravenously into syngeneic mice. A humoral

immune response was then induced by the intraperitoneal immunization of nucleoside conjugated KLH. Suppression of the anti-nucleoside response was assayed by hemolytic plaques 5 days after immunization. Significant suppression was observed for both guanosine and for the tetranucleoside AGCT. The optimal conditions for suppression with nucleoside-coupled spleen cells was found to be a dose of 80×10^6 nucleoside conjugated spleen cells injected i.v. 5 days prior to immunization. The kinetics of suppression showed that the phenomenon is transient, requiring a latent period of 24-48 hours, reaching a peak after 5 days and disappearing by 2 weeks.

The transience of this anti-nucleoside suppression is similar to the transience found in other systems of suppression shown to be mediated by suppressor T cells (77,82,83,87,95). The latent period and transient kinetics of these systems presumably represent the induction and differentiation of suppressor T cells, an active process requiring DNA synthesis. Indeed, Miller, et al. (77) and Scott (84) found that the suppressor mechanism in their system was cyclophosphamide sensitive. These kinetics are hypothesized by Nachtigal, et al. (99) as being due to the short functional life span (approximately 14 days) of immature suppressor cell precursors. Their model describes the immature T_s as either dying within 2 weeks or differentiating into a mature stage after which differentiation into suppressor cells was no longer possible (10). This model was based on experiments in which adult thymectomized mice

were able to be tolerized and produce specific T_s, only up until 14 days after thymectomy. After 14 days, the mice were unable to produce T_s presumably because the pool of thymic T_s precursors was exhausted (10).

Suppression was induced to the immune response against both a T dependent antigen (KLH) in vivo and to a T independent antigen (Ficoll) in vitro. This, together with the kinetic profile of nucleoside suppression is consistent with a suppressor T cell system. Furthermore, much work on the mode or route of administration of haptenated cells by Benacerraf, et al. and others, (82,86) has established that intravenous administration preferentially stimulates suppressor responses. While the same cell preparation adminstered subcutaneously results in delayed type hypersensitivity (82,86), the simultaneous administration of cells by both routes preferentially generates suppressor T cell responses and suppress the development of contact sensitivity (82). The reason for this phenomenon might be the lymphoid tissues the injected cells first encounter: intravenously injected cells tend to localize in the spleen while subcutaneously injected cells localize in lymph nodes (86). Rich, et al. (103) found that after sensitization, spleen cells suppressed MLR responses while lymph node cells enhance MLH. Sy, et al. (100) demonstrated a splenic requirement for induction of suppressor T cells: splenectomy before administration of DNFB did not result in T_s. The spleen was found to be required at least 3 days after tolerization.

The results presented in this thesis are suggestive of T_s involvement but do not definitely prove it. Conclusive proof showing abrogation of suppression with treatment with anti thy 1-2 and complement *in vitro* as well as demonstration of nylon wool enrichment of suppression, are currently in progress. Other experiments planned to further substantiate suppressor T cell involvement include adoptive transfer of splenocytes from mice suppressed with 80 x 10^6 AGCT-SC 5-7 days prior to transfer, as well as *in vitro* studies of the effects of culture supernatants or cell-free extracts on suppression. Hopefully, these data will further elucidate the cellular and biochemical mechanisms of this system.

From the results presented above, it appears that there now exists 2 different techniques for inducing immunological unresponsiveness to nucleosides, the carrier-induced toleranced mechanism described by Borel and Stollar (116) using nucleoside coupled to isologous gammaglobulin and the suppression induced by nucleoside coupled to syngeneic lymphoid cells. The mechanisms of "tolerance" and "suppression" are presumably different. Presenting hapten on soluble protein carriers results in tolerance through receptor blockade of B cells (117). This model postulates that B cells are rendered unresponsive i.e. tolerant, when their antigen receptors are reversibly occupied by the hapten-carrier conjugate (97). Presenting hapten on cell surfaces results in the generation of a clone of antigen-specific suppressor T cells (75,78,79,86). The

precise mechanism of this process is not known. A possible explanation may be that the T_s receptor recognize larger determinants e.g. membrane bound hapten, as opposed to the smaller complex of protein-coupled hapten recognized by B cells. It is well established that antigen cross-reactivity at the T cell level is broader than at the antibody level (118). This difference in cross-reactivity between the T cell receptor and the B cell receptor could account for the exquisite specificity of B cell anti-nucleoside tolerance and the somewhat broader specificity observed for presumed anti-nucleoside T cell suppression.

The mechanism of T cell mediated suppression in the DNFB and the picryl chloride (TNCB) contact sensitivity systems has been extensively investigated and it is now known that T_s can inhibit either the early cell proliferative phase of contact sensitivity (the afferent limb of sensitization) or inhibit the expression of immune effector cells (the efferent limb of sensitization). It is of interest that different haptens / antigens induce T_s that inhibit different limbs of sensitization. Cell proliferation has been shown to be the target of T_s in the suppression of DNFB contact sensitivity (26,119), suppression of MLC responses (120,121), and responses to type III pneumococcal polysaccharide (122). Inhibition of the efferent limb or expression of an immune response has been demonstrated for picryl chloride contact sensitivity (22), DNFB contact sensitivity (25) and response to tumor antigens (123). It is interesting

that the difference of one nitro group (DNFB vs picryl chloride) results in a complete alteration of the mechanism of T_S suppression.

Miller, et al. have recently shown in the DNFB contact sensitivity system, that the suppressor T cells induced by syngeneic DNP-coupled lymphoid cells (syninduced T_S) selectively block the efferent limb of sensitivity by inhibiting the expression of lymph node cells from sensitized animals (25). Co-transferred syninduced T_S block the passive transfer of contact sensitivity to normal recipients. Syninduced T_S did not affect the afferent limb of sensitization and are not H-2 restricted. In contrast, Miller, et al. found that the afferent limb was selectively inhibited by T_S induced by allogeneic DNP-coupled lymphoid cells (alloinduced T_S) leaving the efferent limb unaffected (26). The target of alloinduced T_S appears to be cell proliferation as measured by DNA synthesis. Recipients of alloinduced T_S fail to generate immune lymph node cells capable of transferring contact sensitivity to normal animals. The suppression by alloinduced T_S appears to be H-2 restricted. It is possible that the roles of afferent and efferent blockade in suppressor cell regulation are different: afferent blockade may serve to maintain self-tolerance while efferent blockade may serve to limit clonal expansion after normal immune responses have gotten under way (26). The mechanism of our model of anti-nucleoside suppression has not been dissected sufficiently to determine the precise site of T_S action.

The mechanism of action of suppressor T cells has been found to be mediated by soluble factors in many systems of suppression. Tada et al. (11) characterized an antigen-specific suppressor factor from mice immunized with KLH as smaller than immunoglobulin and containing antigens coded for by the I-J region of the major histocompatability complex (MHC). Kapp and Benacerraf (123) isolated a very similar soluble factor from nonresponder mice primed with GAT which differed in being less MHC restricted in action. Asherson and Zembala (22) described a suppressor factor specific for the picryl chloride contact sensitivity system. It bears H-2 determinants and is selective for the efferent limb of contact sensitivity. The soluble factor isolated for the DNFB contact sensitivity system is also selective for the efferent limb and is H-2 restricted (24,125). The most recently characterized antigen-specific suppressor factor by Taussig, et al. (114) was described in the introduction of this thesis. A number of non-antigen specific factors have also been well characterized. Pierce and Kapp isolated a soluble immune response suppressor (SIRS) from ConA-induced T_s cells which suppress PFC responses non-specifically (6). It does not contain antigens coded by by MHC and has many biochemical similarities to MIF. Rich and Rich isolated a non-specific suppressor factor secreted during mixed lymphocyte reaction (126). This factor bears MHC antigens coded for by the I-C region, and is MHC restricted in action.

The isolation of nucleoside-specific suppressor factor from the anti-nucleoside suppression model would be useful in several ways. It would first substantiate the involvement of T_s cells in the system. It would also allow us to test whether or not suppression of anti-nucleoside antibody can be suppressed without the use of cell bound hapten in vivo. The outcome of this experiment could be significance for new modes of therapy in systemic lupus and other disorders thought to involve T cell suppression.

The syngeneic splenocyte was found to be the most effective carrier for suppression of anti-nucleoside. This is consistent with other models of suppression (77,82,83) confirming the potency of hapten-coupled lymphoid cells as "tolerogen". AGCT-thymocytes suppressed significantly also, Syngeneic erythrocytes and xenogeneic (horse) erythrocytes did not suppress significantly from the control response. The results suggest that the presence of H-2 antigen might be required on the carrier cell surface in order for suppressor T cells to be generated: thymocytes have approximately one fourth the amount of H-2 antigens on spleen cells, and erythrocytes have virtually no H-2 antigen on the membrane (104). This H-2 requirement is consistent with the H-2 requirement well-established for T cell mediated cytotoxicity (105,106) and for some but not all systems of T cell mediated suppression (78,79,84). Further experiments using H-2 incompatible spleen cell from allogeneic strains or H-2 negative cells from tumor cell lines e.g. teratoma cell lines as carriers will be needed to substantiate the H-2 requirement.

The interesting point to consider here is that while no suppression was generated in the nucleoside system by haptenated syngeneic erythrocytes, suppression was evident in other systems (81,87,95,107,108). The hapten used in these other systems was either TNP or DNP and it is possible that the cell surface bound with nucleoside is less tolerogenic than when bound with these compounds. An alternative explanation might simply be that the nucleoside system is H-2 restricted whereas the other systems are not. Further experiments are in progress to examine the effectiveness of administering nucleoside-coupled macrophages, lymph node cells, bone marrow cells, and purified B and T cells. Long and Scott (83) found that thymocytes were "marginally effective" in tolerogenecity, and B cells and bone marrow cell were ineffective.

Using the data for optimal suppression in the C57BL/6 and BDF_1 strains, i.e. 80 x 10^6 AGCT-SC i.v. followed by immunization on day 5, several inbred strains were examined for capacity to generate suppression. All the strains examined: C57BL/6J, DBA/2, BDF_1, (C57BL/6J x DBA/2)F_1, BALB/C could generate suppression except for SJL/J, which did not have significant suppression. It is interesting that SJL are high responders to nucleoside antigens (110) and fail to suppress, while BALB/C are low responders and suppress very well (109). It is possible that SJL lack a T_s clone for regulating the response to nucleic acids, hence decreasing the capacity to generate suppression. There may be other host factors peculiar to SJL involved. SJL mice were recently found to have age-dependent loss of suppressor

ability (111). An interesting study will be to attempt to generate T_s in SJL with AGCT-BALB/C SC, to do the same in BALB/C with AGCT-SJL SC, and to raise suppression in the (BALB/C X SJL/J) F_1 with AGCT-coupled to parental strain SC.

Two findings of this research project are of direct relevance to SLE: 1) the successful generation of suppression in young NZB male mice and BWF, male mice and 2) the specificity of suppression induced by G-SC is broader than the specificity of tolerance induced by G-IgG.

The age dependent loss of suppressor T cell in NZB and NZB/NZW F_1 (BWF_1) mice has been reported occurring between 1 and 2 months of age, according to thymocyte proliferation and antigen-induced suppression assays (112). It is thus very important that suppression was successfully generated in these 8-12 week old mice showing that there is no genetic inability of NZB and BWF_1, mice to generate T_s to nucleosides. Possessing a system of suppression directed against an antibody thought to be the cause of tissue damage in SLE will allow us to test the hypothesis that the pathogenesis of SLE is due to the loss of suppressor T cells. Experiments on the NZW and BWF_1 are in progress and can reveal relevant information on the possible genetic defects in suppression. Sex differences in the generation of suppression will also be examined. If suppressor cells can be successfully generated, it will be important to assess the resultant effect on anti-DNA production as well as the ensuing clinical course of murine lupus.

The finding that the specificity of T cell suppression is

broader than B cell mediated tolerance has obvious therapeutic implications for murine lupus. If oligonucleotides could be coupled to lymphocytes, the resultant suppression could have specificity broad enough to include not just anti-oligonucleotide but antibodies to all nucleic acids including native DNA and RNA.

The broad implications of the nucleoside suppression model in the NZ mice are enormous and will hopefully reveal not only possible therapeutic regimens for human SLE, but elucidate basic cellular mechanisms of immunologic regulation and tolerance.

The basic system of inducing suppression to nucleoside antigens gives us a tool to explore many new areas and happily generates more questions than answers. Can an ongoing immune response to nucleosides be suppressed? Can a secondary response to nucleosides be suppressed? Can cell mediated immunity i.e. cytotoxic "killer" cells or delayed hypersensitivity to nucleoside antigens be generated? What is the effect of suppression induction on the production of anti-DNA? What is the role of the major histocompatability complex in autoimmune disease? Hopefully, the answers to these questions and the important questions related to SLE can be found using the techniques generated in this model.

SUMMARY

A new model of suppression is presented in which suppressor cells to autoantigens i.e. nucleosides have been generated in vivo and in vitro in mice. This was done by covalent coupling of the four nucleosides of DNA to lymphoid cells. The following observations were made:

1) The suppression is dose dependent, requires a latent period to develop and is transient.
2) Anti-nucleoside antibody forming cells to both T dependent and T independent antigens were suppressed.
3) The suppression is antigen-specific but the specificity of suppression (induced by nucleoside-coupled cells) is broader than the specificity of tolerance (induced by nucleoside-coupled gammaglobulin).
4) Anti-nucleoside suppression appears to require H-2 antigen on the cell carrier.
5) Suppression of anti-nucleoside antibody can be generated in a variety of strains of mice except for the SJL/J strain.
6) NZB and BWF_1 mice have the ability to generate suppression to anti-nucleoside antibody.

This model of suppression is significant for the study of systemic lupus because 1) it will allow us to test the hypothesis that the pathogenesis of this disorder is due to the loss of suppressor cells, and 2) the ability to generate nucleoside-specific suppressor cells may prove to be a tool in the treatment of SLE.

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